DP Barcode: D424664 MRID No.: 49513901

DATA EVALUATION RECORD
ACUTE LC_{50} TEST WITH A FRESHWATER INVERTEBRATE
OCSPP 850.1020

1. CHEMICAL: Permethrin PC Code No. 109701

2. TEST MATERIAL: Permethrin TGAI Purity: 97.8%

3. CITATION

Authors:	Brougher DS, Zhang L, Martin KH, Gallagher SP
Title:	Permethrin TGAI: A 96-Hour Flow-Through Acute Toxicity Test with *Hyalella azteca*
Study Completion Date:	November 14, 2014
Laboratory:	Wildlife International, Evans Analytical Group, Easton, MD
Sponsor:	Consumer Specialty Products Association, Washington, DC
Laboratory Report ID:	701A-111A
MRID No.:	49513901
DP Barcode:	D424664

4. REVIEWED BY: John Marton, Ph.D., Environmental Scientist, CDM Smith

Signature:  **Date:** 04/15/15

APPROVED BY: Teri S. Myers, Ph.D., Environmental Scientist, CDM Smith

Signature:  **Date:** 05/15/15

5. APPROVED BY: {……}, {Specialty}, OPP/EFED/ERB-{Section}

Signature: **Date:**

6. DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to freshwater invertebrates. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

7. STUDY PARAMETERS

Age of Test Organism:	10-d old juveniles
Definitive Test Duration:	96 hours
Study Method:	Flow-through
Type of Concentrations:	Time-weighted average (TWA)

8. CONCLUSIONS:

Results Synopsis

96-hour LC_{50}: 6.59 ng ai/L	95% C.I.: 5.40-8.90 ng ai/L
Probit Slope: 4.94	95% C.I.: 2.81-7.08

9. ADEQUACY OF THE STUDY

A. Classification: This study [**is/is not scientifically sound**] and is classified as [**acceptable/supplemental (quantitative)/supplemental (qualitative)/invalid**].

B. Rationale:

C. Repairability:

10. Guideline Deviations: This study was conducted following guidelines outlined in US EPA Series 850- Ecological Effects Test Guidelines, OCSPP 850.1010, *Gammarid Acute Toxicity Test*; and, the ASTM Standard E729-96, *Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians.* The following deviations from OCSPP 850.1020 were noted:

1. *Hyalella azteca* is not a recommended species.
2. Guidance recommends that test organisms not be fed during the test.
3. Due to a biologist's error, the flow rate was 4 vol/24 hours instead of the recommended 5 vol/24 hours.

These deviations do/do not impact the acceptability of the study.

11. SUBMISSION PURPOSE: This study was submitted to provide data on the effects of permethrin on survival of the freshwater amphipod (*Hyalella azteca*) following acute exposure for the purpose of chemical re-registration

12. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species is *Daphnia magna*	*Hyalella azteca*
All organisms are approximately the same size and weight?	Yes
Life Stage Daphnids: 1st instar (<24 h). Amphipods, stoneflies, and mayflies: 2nd instar. Midges: 2nd & 3th instar.	10-day old juveniles
Supplier	In-house cultures. Culture stock originally obtained from Aquatic BioSystems, Fort Collins, CO.
All organisms from the same source?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 7 days	Culture stock continuously maintained under conditions comparable to test conditions.
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	No
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A

Guideline Criteria	Reported Information
Feeding No feeding during the study.	Amphipods were fed daily during the acclimation with Tetramin® flake food. During the test, amphipods in each test chamber received 1 mL of a mixture of yeast, cereal grass media, and trout chow (YCT) at approximately 48 hours.
Pretest Mortality No more than 3% mortality 48 hours prior to testing.	None reported

C. Test System:

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, **not** dechlorinated tap water.	On-site well water was passed through a sand filter (~ 25 µm), aerated, filtered to 0.45 µm, and UV-sterilized.
Does water support test animals without observable signs of stress?	Yes
Water Temperature Daphnia: 20EC Amphipods and mayflies: 17EC Midges and mayflies: 22EC Stoneflies: 12EC	22.82-23.01°C
pH Prefer 7.2 to 7.6.	7.5-8.1
Dissolved Oxygen Static: ∃ 60% during 1st 48 h and ∃ 40% during 2nd 48 h, flow-through: ∃ 60%.	6.9-8.6 mg/L (lowest reading in solvent control at 96 hours) ≥80% of saturation maintained throughout test
Total Hardness Prefer 40 to 48 mg/L as $CaCO_3$.	144 mg/L as $CaCO_3$

Guideline Criteria	Reported Information
Test Aquaria 1. Material: Glass or stainless steel. 2. Size: 250 ml (daphnids and midges) or 3.9 L (1 gal). 3. Fill volume: 200 ml (daphnids and midges) or 2-3 L.	1. Glass beaker inside of Teflon-lined stainless steel aquaria 2. 300 mL beakers, 25 L aquaria 3. 23 L of water in aquaria Nylon mesh screening covered two circular holes (~2.5 cm in diameter) on opposite sides of the beakers. Four 2x2 cm squares of nylon mesh were placed in the bottom of each beaker to serve as a substrate for the amphipods.
Type of Dilution System Must provide reproducible supply of toxicant.	Continuous-flow diluter. Syringe pumps delivered volumes of test substance stock solutions to mixing chambers, and the solvent (DMF) was delivered to a separate mixing chamber for the solvent control. These solutions were mixed with well water in mixing chambers prior to delivery to the test chambers. Flow was controlled using rotameters. Delivery of the test water to the chambers was initiated 19 days prior to introduction of test organisms and the general operation was checked visually at least once on the first and last days of the test and at least twice daily during the test.
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.	Approximately 4 vol/24 hours

Guideline Criteria	Reported Information
Biomass Loading Rate Static: # 0.8 g/L at # 17°C, # 0.5 g/L at > 17°C; flow-through: # 1 g/L/day.	Not reported
Photoperiod 16 hours light, 8 hours dark.	16L:8D with 30-minute transition periods of low-light intensity
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests.	DMF (0.1 mL/L)

D. Test Design:

Guideline Criteria	Reported Information
Range Finding Test If LC_{50} >100 mg/L, then no definitive test is required.	Nominal concentrations were selected in consultation with the study sponsor based on results from preliminary range-finding data. No further details were provided.
Nominal Concentrations of Definitive Test Control & 5 treatment levels; a geometric series with each concentration being at least 60% of the next higher one.	0 (negative and solvent controls), 3.8, 7.5, 15, 30, and 60 ng ai/L
Number of Test Organisms Minimum 20/level, may be divided among containers.	20/level with 10/replicate
Test organisms randomly or impartially assigned to test vessels?	Yes

Guideline Criteria	Reported Information
Water Parameter Measurements 1. Temperature Measured continuously or, if water baths are used, every 6 h, may not vary > 1°C. 2. DO and pH Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control.	1. Temperature was measured in each test vessel at 0 and 96 hours. Temperature was also continuously measured in the negative control. 2. DO and pH were measured at test initiation and every 24 hours thereafter. When 100% mortality occurred, temperature, DO, and pH were measured and then discontinued.
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Samples were collected for analytical verification at 0, 48, and 96 hours.

13. REPORTED RESULTS:

Guideline Criteria	Reported Information

Quality assurance and GLP compliance statements were included in the report?	Yes. Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided. This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency (40 CFR Parts 160 and 792); OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and, Japan MAFF (11 NohSan, Notification No. 6283, Agricultural Production Bureau, 1 October 1999), with the following exception: periodic analyses of water for potential contaminants were not performed according to Good Laboratory Practice Standards, but were performed using a certified laboratory and standard US EPA analytical methods.
Control Mortality Static: #10% Flow-through: #5%	0% in both the negative and solvent controls
Percent Recovery of Chemical	Mean-measured concentrations yielded recoveries of 38 to 65% of nominal with coefficients of variation of 7.51 to 47.1%. The reviewer-calculated TWA concentrations yielded recoveries of 37.8 to 64.5% of nominal with CVs of 7.5 to 47.1%.
Raw data included?	Yes

Mortality

Concentration (ng ai/L)		Number of Organisms	Cumulative Number Dead			
Nominal	Mean Measured/ TWA		Hour of Study			
			24	48	72	96
Control	--	20	0	0	0	0
Solvent Control	--	20	0	0	0	0
3.8	2.0/1.8	20	0	0	0	0
7.5	3.6/3.6	20	0	0	0	3
15	5.8/5.7	20	0	0	0	6
30	19/19	20	20	20	20	20
60	34/34	20	20	20	20	20

Mortality first occurred after 24 hours in the top two treatment groups (100% mortality), then no additional mortalities occurred under the last observation. The study authors reported a 96-hr LC_{50} of 6.7 (5.5-8.9) ng ai/L, with a probit slope of 5.0, based on the mean-measured concentrations.

Other Significant Results: All amphipods in the controls and the lowest treatment level appeared normal and healthy throughout the test, exhibiting no signs of intoxication or impaired behavior. Organisms in these groups were occasionally observed to be floating at the solution surface, but appeared normal once gently submerged; observations of floating organisms were considered to be incidental to treatment. Complete mortality occurred in the top two treatment groups by 24 hours, and lethargy was observed among amphipods in the mean-measured 3.6 and 5.8 ng ai/L treatment groups.

B. Statistical Results

Method: The 96-hr LC_{50} value was estimated via the probit analysis using the computer program of C.E. Stephan. Toxicity values were based on the mean-measured concentrations.

96-hour LC_{50}: 6.7 ng ai/L 95% C.I.: 5.5-8.9 ng ai/L

Probit Slope: 5.0

14. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Untrimmed Spearman-Kärber LC_{50} (95% C.I.)	7.44 (6.13-9.03) ng ai/L
Probit LC_{50} (95% C.I.)	6.59 (5.40-8.90) ng ai/L
Probit Slope (95% C.I.)	4.94 (2.81-7.08)

The reviewer estimated the 96-hr LC_{50} and 95% C.I. using the probit analysis via CETIS statistical software version 1.8.7.12 with database backend settings implemented by EFED on 3/25/14. Toxicity values were estimated using the reviewer-calculated TWA concentrations.

15. REVIEWER'S COMMENTS:

The reviewer's results were based on the TWA concentrations, whereas the study authors used the mean-measured concentrations. Therefore, the reviewer's results are reported in the Conclusions section of this DER.

Analytical recoveries at the lowest treatment level were <LOQ at all sampling periods. However, peaks were detected and the concentrations were estimated via extrapolation. The reviewer used these extrapolated values in calculated the time-weighted average concentrations using the following equation:

$$C_{TWA} = \frac{\left(\frac{C_1 + C_0}{2}\right)(t_1 - t_0) + \left(\frac{C_2 + C_1}{2}\right)(t_2 - t_1) + \left(\frac{C_{n-1} + C_2}{2}\right)(t_{n-1} - t_2) + \left(\frac{C_n + C_{n-1}}{2}\right)(t_n - t_{n-1})}{t_n}$$

where:
C TWA is the time-weighted average concentration,
C_j is the concentration measured at time interval j (j = 0, 1, 2,...n)
t_j is the number of hours (or days or weeks, units used just need to be consistent in the equation) of the test at time interval j
(e.g., t_0 = 0 hours (test initiation), t_1 =24 hours, t_2 =96 hours)

Recoveries of the test material over the course of the test were low (37.8-64.5% of nominal based on TWA concentrations) with high variability (CVs of 7.5-47.1%). However, the study

authors reported that based on the physic-chemical properties of permethrin under exposure conditions, the analytical recoveries and variability were expected and consistent with past behavior observed with pyrethroids in aqueous systems. The exposure system surface area, biomass, and the addition of food all likely contributed to sorptive losses. Further, the analytical results were considered representative of results in previous testing and were therefore reasonable given the anticipated behavior of permethrin under test conditions.

The in-life portion of the definitive toxicity test was conducted from August 11 to 15, 2014.

This study [**is/is not scientifically sound**] and is classified as [**acceptable/supplemental (quantitative)/supplemental (qualitative)/invalid**].